Cold-wet Imbibition Injury in Beans $\frac{1}{2}$

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The problem of poor stands and low yields following emergence under cold-wet imbibition conditions has long plagued bean (Phaseolus vulgaris) growers and breeders. For a time cold was thought to be the principle deterrent factor, especially after Pollock (1965) showed that beans go through a temperaturesensitive stage during imbibition. In 1969 Pollock also showed that initial seed moisture was an important variable. Various workers (Orphanos and Haydecker 1968, Powell and Matthews 1978, and Roos and Pollock 1971) have shown that soaking injury is a critical factor, but it was often assumed that low oxygen availability due to flooding was the key to subsequent injury. Silbernage1 (1977) reported that a black-seeded selection out of the USDA Plant Introduction accession number 165426 was tolerant to emergence under cold-wet imbibition conditions. However no one had examined all the variables concurrently on the same varieties using uniformly high-quality seed to eliminate the variability due to mechanical injury. Mechanical injury effects can mask the effects of other stresses (Silbernagel and Burke 1973). In view of the uncertainty expressed in the literature, we thought it would be useful to systematically define the individual and combined effects of differences in bean genotype, initial seed moisture content, temperature, and oxygen content during the first 48 hours of bean seed imbibition.

A stress sensitive cv. Goldcrop and the stress tolerant P.I. 165426-BS were imbibed in a sand medium at 10 vs. 22° C, 8 vs. 12% initial seed moisture, and 0, 1, 2, 5, and 21% 0 content. Submersion for various times in water was used to induce "flooding" injury.

At 22°C, low 0_2 concentrations of 0, 1, and 2% increased leaching from seeds, delayed emergence, and reduced growth compared to 5 and 21% 0_2 . Adverse emergence and growth effects (Table 1) were aggravated by 8% (vs. 12%) initial seed moisture content, especially in the sensitive 'Goldcrop'. At 10° C the effect of 0_2 deficiency was minimal. Low temperature inhibited growth of the sensitive 'Goldcrop' and increased leaching from both genotypes. Lowering initial seed moisture from 12 to 8%, reduced survival of seeds of both genotypes exposed to 10° C. Generally P.I. 165426-BS was not as sensitive to any of the stresses or combination of stresses as 'Goldcrop'. Therefore the potential for improvement through breeding for stress resistance seems promising.

Flooding of seeds with water for 24 h increased leaching and reduced emergence and growth much more than 24 h replacement of 0_2 with nitrogen. Since some symptoms of chilling and flooding during imbibition are similar, it is suggested that the mechanisms of these injuries are related. Our results

 $[\]frac{1}{E}$ Excerpted from WSU-MS thesis of senior author: "Oxygen and temperature effects on germination of two bean genotypes at two seed moisture levels."

support those of others which indicate that factors other than oxygen deficiency are involved in flooding injury, because: a) Flooding injury was much more severe than the injury caused when 02 was replaced with N2 (Table 2); b) Low temperature aggravated flooding injury but reduced the detrimental effect of low oxygen (Table 1, figs. 2 and 4); c) Low seed moisture increased the sensitivity of seeds to flooding and increased flood related leaching (Bramlage et al., 1978). However, low seed moisture did not increase leaching as a result of oxygen deficiency in either genotype and did not effect the sensitivity of the tolerant P.I. 165426-BS to oxygen stress (Table 1, fig. 2); and d) Flooding results in severe damage during the first one-third of the imbibition period. The effect of oxygen starvation is likely to await the onset of seedling metabolism when anaerobically induced ethanol production can damage membranes (Crawford 1977, McManmon and Crawford 1971).

Faulty membrane reorganization at low temperatures has been suggested as the cause of chilling injury to imbibing seeds (Bramlage 1978). Since imbibitional chilling injury is irreversible in peas and cottonseed (Christiansen and Thomas 1969, Highin and Lang 1966), and only partly reversible in lima beans (Pollock 1969), it may be that membranes of seeds imbibed at low temperatures cannot reorganize normally even after the temperature increases. In this saturated condition, perhaps membrane phospholipids cannot form a continuous layer across the relatively large quantities of water. Thus, membrane reorganization may be restricted to relatively low levels of seed moisture content. A corollary can be seen in flooding effects: flooding injury was reported to correlate with the rate of imbibition and with the final amount of water absorbed by the seeds (Barton and McNab 1956). This supports a hypothesis that too rapid imbibition prevents membrane reorganization because the seed moisture content quickly exceeds a level which permits membrane reorganization. According to this hypothesis, flooding injury would be more severe in dry seeds because more extensive membrane reorganization has to take place. Rapid imbibition in high moisture seeds would cause only slight damage because of a high level of preimbibitional membrane reorganization. At low temperatures the injury may be intensified because low temperature prevents even partial membrane reorganization.

Oxygen deficiency had a pronounced effect on electrolyte leaching (fig. 3) and a relatively small effect on leaching of sugars (fig. 4), whereas temperature stress had a relatively large effect on leaching of sugars as compared to that of electrolytes. This qualitative difference in leaching response may indicate that $\mathbf{0}_2$ stress incites a different leaching mechanism than low temperature stress does. The sugar molecule is relatively large compared to electrolyte molecules. Since temperature stress caused high sugar leakage, larger membrane discontinuities may have resulted from low temperature than from low oxygen (Bramlage 1978, Simon 1974).

The magnitude of leaching may influence the extent of seed and seedling infection by fungal pathogen, because sugars and other organic compounds serve as substrates for these fungi and stimulate their pathogenic activity (Hayman 1969, Schroth et al., 1966). Since low temperature and flooding both induced considerable leaching of sugars from seeds, it seems reasonable to expect these stresses to lead to increased severity of seed and seedling rot in sensitive cvs, and a "breakdown" of resistance in tolerant cvs. If this reasoning is valid, then breeders should combine stress tolerance with pathogen tolerance to prevent a breakdown of resistance under adverse environmental conditions.

Table 1. Emergence from 8% and 12 m isture seeds of two genotypes, as affected by five oxygen concentrations and two temperatures during a 48 hour imbibition period.

		Goldcrop				PI-165426-BS					
	Seed Moisture - %										
	8			12	8		12				
%	Temperature - C										
02	10	22	10	22	10	22	10	22			
0 1 2 5 21	4.8 4.8 4.9 4.8 4.8	4.6 4.8 3.8 2.6 2.5	-days from 4.7 4.7 4.6 4.7 4.7	3.6 3.7 2.6 2.0 1.9	4.1 4.1 4.2 4.0 4.2	% emergence 3.0 2.9 2.4 2.2 2.0	3.7 3.5 3.6 3.6 3.6	3.0 2.9 2.3 1.7			

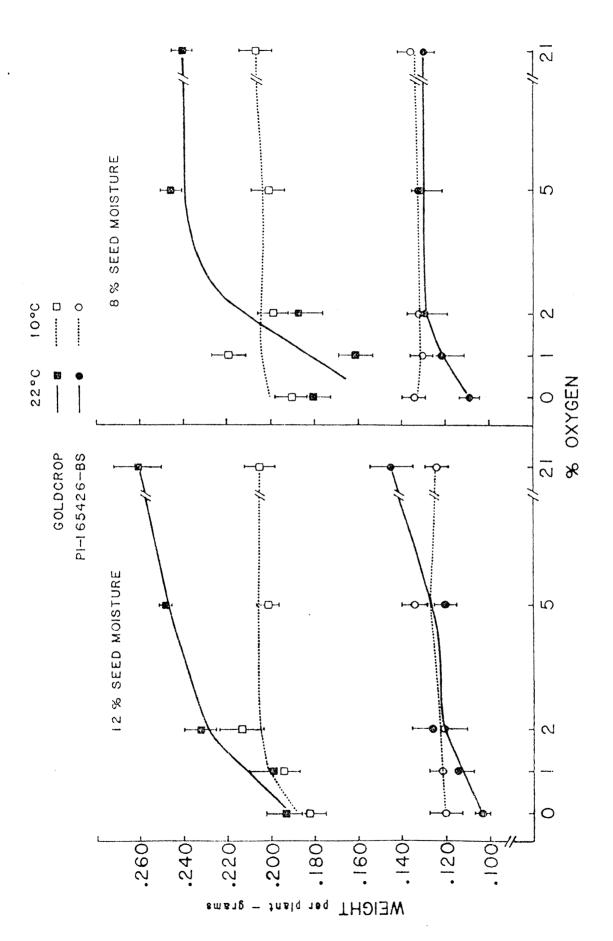
Table 2. Effects of oxygen deficiency and flooding during the first 24 hours of germination of Goldcrop with 11% seed moisture.

	Seed	Lea	Leaching			Seedling	
	weight	equiv	equivalents of			top	No rma l
Treatment	increase	, ксі	Clucose	emergence	Emergencex	weights ^X	plants ^x
	percent	рд	μg/seed			g/plant	percent
Air	105.7±.3 ^z	144.0± 9.9	21.3± 1.6	1.9±.07	100	.234±.004	98
NItrogen	91.8±.2	411.2±24.1	79.2±12.7	2.8±.04	100	.208±.006	98
Flood	105.4±.8	1023.4±11.4	312.9± 8.9	3.8±.16	92	.129±.006	46

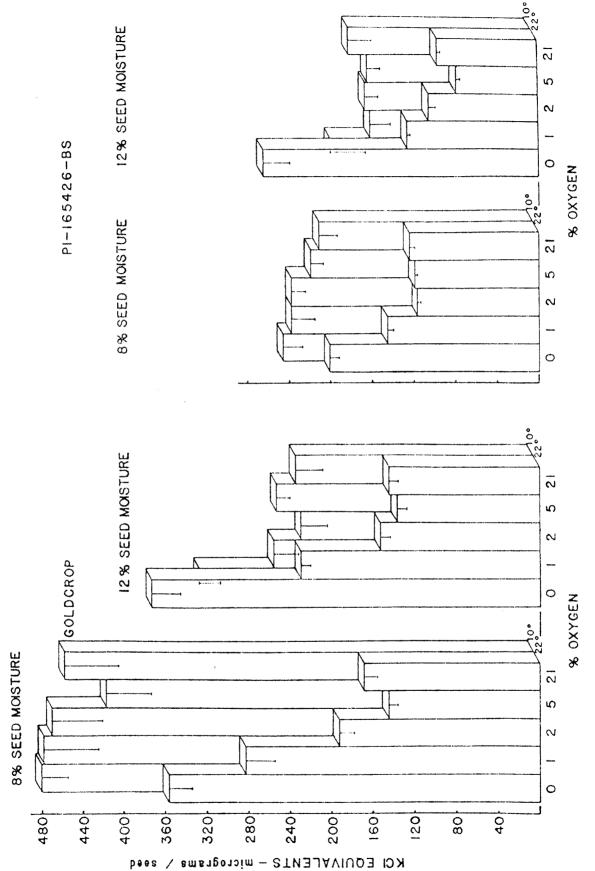
 $^{^{\}rm Z}_{\rm Means}$ and standard error of the mean.

yWeight after the 24-hour treatment period as a percent of beginning seed weight

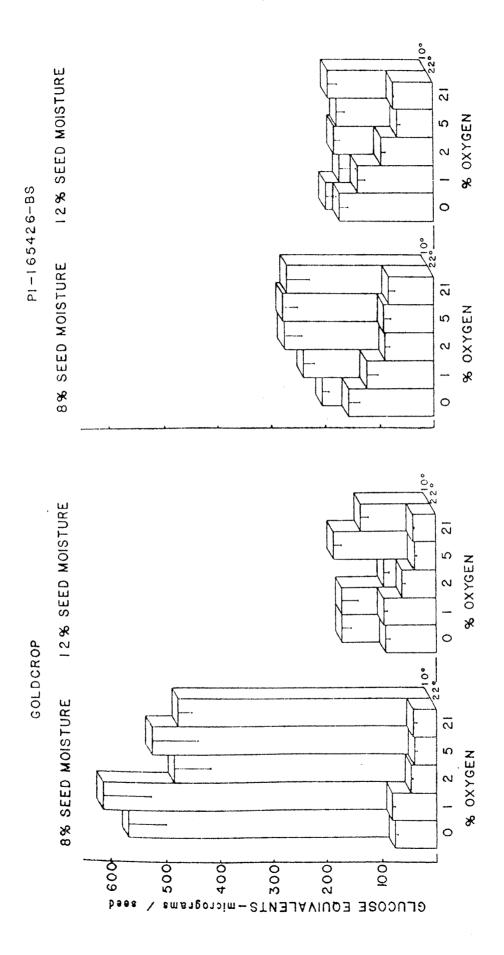
x_{Ten days after emergence.}



Dry weights of 10-day-old seedlings from 8% and 12% moisture seeds of two genotypes, exposed to five oxygen levels and two temperatures (barmsess and error of the mean). Fig. 2.



Electrolyte leachate from 8% and 12% moisture seeds of two genotypes exposed to five oxygen levels and two temperatures (bar=standard error of the mean). Fig. 3.



Sugars and related substances leached from 8% and 12% moisture seeds of two genotypes exposed to five oxygen levels and two temperatures (bar=standard errors of the mean). Fig. 4.